Persistent parvovirus B19 infections with different clinical outcomes in renal transplant recipients: diagnostic relevance of polymerase chain reaction (PCR) and of quantification of B19 DNA in sera

Alberta Azzi[1], *Krystyna Zakrzewska*[1], *Elisabetta Bertoni*[2], *Stefano Guidi*[3] *and Maurizio Salvadori*[2]

[1]Institute of Microbiology, University of Florence, Florence, Italy; [2]Department of Renal Transplantation and [3]Department of Hematology, Careggi Hospital, Florence, Italy

Objective: To study parvovirus B19 infection in immunocompromised subjects such as renal transplantation recipients.

Methods: Two cases of B19 infection in renal transplant recipients have been included in the study. The outcome of the infection has been studied by both serologic and virologic methods. A monitoring of the DNAemia was done by a nested PCR in endpoint titration assays.

Results: In one patient with severe anemia an acute B19 infection was diagnosed by PCR 26 days after the transplant; a high level of DNAemia persisted until an intravenous immunoglobulin treatment. Then a sharp decrease of the DNAemia was shown, without full clearance of B19 virus. In a lymphocyte suspension from the organ donor, B19 DNA was detected. In the other patient, who recovered spontaneously from anemia, a persistent B19 infection was demonstrated at day 106 after transplantation and was still demonstrable after 470 days.

Conclusions: A high level of B19 DNAemia was associated with symptomatic infection, with severe anemia, whereas low-level DNAemia was long-lasting in asymptomatic subjects with impaired immunologic responses. The endpoint titration assay by nested PCR was very useful for the monitoring of B19 infection, particularly following the therapeutic intravenous immunoglobulin administration.

Key words: Parvovirus B19, renal transplantation, persistent B19 infection, semiquantitative PCR

Twenty years after its discovery in sera from asymptomatic subjects [1], the human parvovirus B19 is known to be the cause of several pathologic conditions, ranging from erythema infectiosum to transient aplastic crisis or hydrops fetalis [2–5]. In immunocompetent people, acute B19 infections generally occur, but it is now well documented that in immunocompromised subjects B19 virus can persist, leading to severe acute or chronic anemia [5–8]; moreover, two cases of persistent B19 infections in immunocompetent subjects have also been described [9–11].

Corresponding author and reprint requests:

Alberta Azzi, Istituto di Microbiologia, Università di Firenze, Viale Morgagni 48, 50134 Firenze, Italy

Tel: 39 55 411081 Fax: 39 55 4223895

Accepted 29 February 1996

Renal transplantation recipients should be considered at risk of B19 infection because of the immunosuppressive treatments; however, two cases only of B19 infection in such patients have been reported up to now. The first observation dates back to 1986 [12] and concerns a case of B19 infection associated with a transient bone marrow dysfunction, 1 month after the transplantation. The second observation described in 1993 [13] concerns a case of B19 infection associated with pure red cell aplasia and peripheral pancytopenia, which occurred 1 year after the renal transplant. In addition, one of the sera first detected as B19 virus positive by Cossart et al. [1] was from a patient 1 week after renal transplantation. It is probable that the rate of B19 infection in such patients is underestimated, because B19 virus is so far not sufficiently known worldwide.

Here we report two cases of B19 infection in two renal transplant recipients, characterized by different clinical outcomes, detected simultaneously in the spring of 1994. A semi-quantitative application of the nested polymerase chain reaction (PCR) allowed the monitoring of the virologic outcome of B19 infection, especially in a patient who required an intravenous immunoglobulin (IVIG) treatment.

MATERIALS AND METHODS

Patients and samples examined

Two patients, TF, a 53-year-old man, and BR, a 47-year-old woman, under-went a cadaveric kidney transplantation on 1 January 1994 and 21 February 1994, respectively, at the Department of Renal Transplantation of the Careggi Hospital in Florence. The transplant was successful in both patients, with good primary function. The immunosuppression included prednisone and cyclo-sporin. TF received in addition azathioprine from day 11 after the transplant, but this additional treatment was then stopped because of an inhibitory effect on either the erythroid or the myeloid compartment of the bone marrow; nevertheless the anemia persisted. Bladder bleeding occurred 4 days after the transplant in patient BR, and two transfusions of packed red blood cells were required. A few days later, the patient developed a severe hypoplastic anemia (hematocrit 15.2%, reticulocyte count 0.2–0.5%). The bone marrow was hypo-cellular with giant pronormoblasts. The patient was treated at first with IVIG (200 mg/kg) twice a week, without clinical improvement; therefore, on day 56 after transplant, the doses were increased to 400 mg/kg per day for 15 days. A few days after the start of this treatment, an increase of the reticulocyte count and the resolution of the anemia were observed.

A series of 20 serum samples were drawn from patient BR, on days 26 to 380 after transplantation, for the diagnosis and monitoring of B19 infection; in addition, a serum sample taken before transplantation was available. Moreover, a bone marrow sample at day +66 and two throat swabs (day +56 and day +94) were also obtained from the same patient for B19 DNA detection. A series of four sera were drawn from TR, on days 103 to 391 after renal transplantation.

Finally, lymphocytes from the two kidney donors were also analyzed for B19 DNA presence, and a serum sample from a blood donor, 1 month after the donation to BR, was analyzed for B19 DNA and for anti-B19 IgM and IgG.

Virologic and serologic methods

A dot-blot hybridization assay with a digoxigenin-labeled probe [14] and a nested PCR [15] have been used for B19 DNA detection. In order to eliminate inhibitors of the Taq polymerase, serum samples were heated at 72 °C for 45 s, whereas DNA from the bone marrow sample (200 μL) and the throat swabs (400 μL) was extracted by the phenol/chloroform method, after digestion with proteinase K. A semiquantitative PCR was also used to evaluate the concentration of B19 DNA in the sera at different times during the infection. An endpoint titration method, with a cloned B19 DNA as an external standard, was used: the B19 DNA sequence, 1112 bp long, amplified by the external primers in the nested PCR, was cloned in the plasmid pGEM-T, which was used to transform *E.coli* JM 109. This plasmid was subsequently employed as external B19 DNA standard in the nested PCR. Dilutions containing 0.71 fg, 0.071 fg, 0.0071 fg and 0.00071fg of the B19 DNA target and 10-fold serial dilutions of the sera to be titered were used in the PCR assay. The endpoint was given by the highest dilution of the standard DNA which resulted in a visible band after electrophoresis in agarose gel stained with ethidium bromide (0.5 μg/mL). Triplicate assays were carried out.

Appropriate positive and negative controls were performed in each PCR, and guidelines to prevent false-positive results were adopted. Pretreatments of sample, preparation of PCR mix, addition of the samples to the mix, PCR cycling and electrophoresis were performed in five different laboratories.

Two commercial kits for anti-B19 IgM and IgG detection were used: the kit from Ferring using a synthetic peptide as antigen, and the kit from Dako using a recombinant antigen obtained in a baculovirus–insect cell expression system and an antibody capture assay for anti-B19 IgM detection. The results were always concordant.

RESULTS

Only the serum samples drawn from the patient BR before the beginning of the treatment with high doses of IVIG gave positive results in the dot-blot hybridization assay with a B19 DNA-specific probe, digoxigenin labeled, whereas by the nested PCR all serum samples from patient BR, as well as four out of five serum samples from patient TR, obtained after renal transplantation, were positive for B19 DNA. However, the use of a semiquantitative PCR showed that the viral load in these samples was very different (Table 1).

In fact, a high-level DNAemia was demonstrable in the first serum sample available, day +26, and persisted until day +57, when the treatment with high doses of IVIG began. At day +60 a decrease of B19 DNAemia was already evident. However, B19

Table 1 Monitoring of B19 infection by B19 DNA and anti-B19 antibody detection in series of sera from the two patients

		Days after transplant											
Patient	Assay	26	36	57	60	65	74	92	106	121	204	229	380
BR	PCR	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	pos.	nt	nt	pos.
	lim. dil	10^{-9}	10^{-6}	10^{-9}	10^{-3}	undil.	undil.	undil.	undil.	undil.	nt	nt	undil.
	no. part	10^{10}	10^{7}	10^{10}	10^{4}	10	10	10	10	10	nt	nt	10
	IgM[a]	+++	+++	+++	+++	+++	+++	+++	+++	+++	nt	nt	+++
	IgG[b]	−	+	+	++	+++	+++	+++	++	++	nt	nt	−
TR	PCR	nt	nt	nt	nt	nt	nt	nt	pos.	nt	pos.	pos.	neg.
	lim. dil.	nt	nt	nt	nt	nt	nt	nt	undil.	nt	undil.	undil.	neg.
	no. part	nt	nt	nt	nt	nt	nt	nt	10	nt	10	10	neg
	IgM[a]	nt	nt	nt	nt	nt	nt	nt	+++	nt	+++	+++	+++
	IgG[b]	nt	nt	nt	nt	nt	nt	nt	+	nt	+	+	−

[a]Anti-B19 IgM; [b]Anti-B19 IgG.
lim. dil = limiting dilution; no. part. = number of viral particles; +++ = strongly positive; ++ = positive; + weakly positive, undil. = undiluted; nt = not tested.

DNAemia persisted at very low concentration and was still demonstrable more than 1 year after the transplant. In contrast, in the patient TF the level of B19 DNAemia was steadily low in the first three serum samples; in a sample drawn more than 1 year after transplantation, B19 DNA was undetectable by the nested PCR, but it was again positive in the last sample available 570 days after transplantation.

In addition, a bone marrow sample, taken from patient BR at day +66, was morphologically normal, but PCR positive for B19 DNA.

A characteristic pattern of the antibody response was observed in both patients: in patient BR, anti-B19 IgG was still negative in the first sample examined after transplantation, and low IgG levels were detectable until the IVIG administration at high dose; following this treatment, in fact, high levels of anti-B19 IgG appeared and were still detectable in a patient serum at day +92. A slight decrease was detectable in sera at days +106 and +121 and, 1 year later, the anti-B19 IgG was again undetectable, whereas the anti-B19 IgM persisted at high levels, unmodified from the beginning of the infection. The patient TR also presented constant high levels of anti-B19 IgM but very low levels of IgG, which in the last two samples was undetectable.

Both patients were B19 antibody negative before transplantation, and B19 DNA was absent in sera drawn at the time of the transplant.

In order to investigate the source of B19 transmission, lymphocyte suspensions from the two kidney donors, stored frozen under liquid nitrogen, were tested for B19 DNA presence: the lymphocytes of the young donor to the patient BR gave weakly positive results in the nested PCR for B19 DNA, whereas the lymphocytes from the donor to the patient TR gave negative results. To exclude the possibility of a false-positive result due to contamination of the PCR, the lymphocyte suspension of BR stored in two different vials has been analyzed at different times.

With the aim of assessing the possibility of respiratory shedding of B19 virus by patient BR, and thus the risk of transmission of the infection to hospital staff [16] or other contacts, during the highly viremic phase a throat swab at day +56, from a little ulcerative lesion, was tested for B19 DNA and was PCR positive, whereas the throat swab at day +94 was negative.

DISCUSSION

The results reported here confirm that immunodepressed subjects are at risk of persistent B19 infection which may cause anemias of different severity. In fact, in one case only high doses of IVIG were required for clinical recovery. The different clinical outcome of B19 infection in these two patients could be related to different routes of transmission and to different viral loads transmitted or, in addition, to different host conditions at the time of the infection, which are unknown in TR's case.

In both patients, however, B19 infection persisted after the recovery from anemia, as shown by the persistence of very low levels of DNAemia.

B19 DNA was demonstrated in a lymphocyte suspension from the kidney donor of the first patient (BR). It is likely that residual B19 DNA from the whole blood was still present in the lymphocyte suspension, so that it could be detected by the highly sensitive nested PCR. The lymphocyte suspension from the kidney donor of the patient TR was B19 DNA negative.These results strongly suggest that B19

infection has been transmitted to the patient BR in the transplanted organ, but do not allow us to exclude a role of the transplanted kidney in the transmission of B19 infection to TR, even if it seems more likely that this patient became infected by the common respiratory route.

As previously reported, dot-blot hybridization assay was able to detect B19 viremia during the acute phase only, when high amounts of B19 particles were free in the blood, but failed to demonstrate persistent infections characterized by very low viral load [17,18]. Indeed, persistent B19 infection could be shown by the nested PCR only, which could detect 10 viral genomes.

The nested PCR, however, used as a qualitative assay cannot differentiate between acute and late or persistent infections and can neither identify differences in virus concentration which could be related to symptomatic or asymptomatic conditions nor indicate a level of viremia that may warrant initiation of antiviral therapy [19]. A number of different quantitative or semi-quantitative PCR applications have been developed in this regard: competitive methods based on the use of an internal standard are at present considered the most accurate quantitative assays; however, the very simple and traditional method of endpoint titration may be a suitable alternative for detecting wide variation in the amount of target DNA sequences in series of specimens taken for monitoring virus infection following therapeutic treatments [20].

In this study the use of the nested PCR in endpoint titration assays for monitoring B19 infection allowed us to assess the early therapeutic effect of IVIG. In addition, the results suggest that a low-level B19 viremia, of about 5000 viral particles/mL, may be associated with persistent asymptomatic B19 infection.

Acknowledgements

Krystyna Zakrzewska was supported by a fellowship from the Foundation 'Istituto di ricerche virologiche Oretta Bartolomei Corsi'.

References

1. Cossart YE, Field AM, Cant B, Widdows D. Parvovirus-like particles in human sera. Lancet 1975; i: 72–3.
2. Anderson MJ, Jones SE, Fisher-Hoch SP, et al. Human parvovirus, the cause of erythema infectiosum (Fifth disease)? [letter]. Lancet 1983; i: 1378.
3. Pattison JR, Jones SE, Hodgson J, et al. Parvovirus infections and hypoplastic crisis in sickle-cell anaemia [letter]. Lancet 1981; i: 664–5.
4. Anand A, Gray ES, Brown T, Clewley JP, Cohen BJ. Human parvovirus infection in pregnancy and hydrops fetalis. N Engl J Med. 1987; 316: 183–6.
5. Brown KE, Young NS, Liu JM. Molecular, cellular and clinical aspects of parvovirus B19 infection. Crit Rev Oncol Hematol 1994; 16: 1–31.
6. Kurtzman GJ, Ozawa K, Cohen B, Hanson G, Oseas R, Young NS. Chronic bone marrow failure due to persistent B19 parvovirus infection. N Engl J Med 1987; 317: 287–294.
7. Azzi A, Macchia PA, Favre C, et al. Aplastic crisis caused by B19 virus in a child during induction therapy for acute lymphoblastic leukemia. Haematologica 1987; 74: 191–4.
8. Frickhofen N, Abkowitz JL, Safford M, et al. Persistent B19 parvovirus infection in patients infected with human immunodeficiency virus type 1 (HIV-1): a treatable cause of anemia in AIDS. Ann Intern Med 1990; 113: 926–33.
9. Faden H, Gary GW, Anderson LJ. Chronic parvovirus infection in a presumably immunologically healthy woman. Clin Infect Dis 1992; 15: 595–7.
10. Cassinotti P, Schultze D, Schlageter P, Chevili S, Siegl G. Persistent human parvovirus B19 infection following an acute infection with meningitis in an immunocompetent patient. Eur J Clin Microbiol Infect Dis 1993; 12: 701–4.
11. Sasaki T, Murai C, Muryoi T, Takahashi Y, Munakata Y, Abe K. Persistent infection of human parvovirus B19 in a normal subject [letter]. Lancet 1995; 346: 851.
12. Neild G, Anderson M, Hawes S, Colvin BT. Parvovirus infection after renal transplant [letter]. Lancet 1986; ii: 1226–7.
13. Corral DA, Darras FS, Jensen CWB, et al. Parvovirus B19 infection causing pure red cell aplasia in a recipient of pediatric donor kidneys.Transplantation 1993; 55: 427–9.
14. Azzi A, Zakrzewska K, Gentilomi G, Musiani M, Zerbini M. Detection of B19 parvovirus infections by a dot blot hybridization assay using a digoxigenin-labelled probe. J Virol Methods 1990; 27: 125–34.
15. Zakrzewska K, Azzi A, Patou G, Morfini M, Rafannelli D, Pattison JR. Human parvovirus B19 in clotting factor concentrates: B19 DNA detection by the nested polymerase chain reaction. Br J Haematol 1992; 81: 407–12.
16. Bell LM, Naides SJ, Stoffman P, Hodinka RL, Plotkin SA. Human parvovirus B19 infection among hospital staff members after contact with infected patients. N Engl J Med 1989; 321: 485–91.
17. Musiani M, Azzi A, Zerbini M, et al. Nested polymerase chain reaction assay for the detection of B19 parvovirus DNA in human immunodeficiency patients. J Med Virol 1993; 40: 157–60.
18. Hornsleth A, Carlsen KM, Christensen LS, Gundestrup M, Heegard ED, Myhre J. Estimation of serum concentration of parvovirus B19 DNA by PCR in patients with chronic anaemia. Res Virol 1994; 145: 379–86.
19. Rawal BK, Booth JC, Fernando S, Butcher PD, Powles RL. Quantification of cytomegalovirus DNA in blood specimens from bone marrow transplant recipients by the polymerase chain reaction. J Virol Methods 1994; 47: 189–202.
20. Kulski JK. Quantitation of human cytomegalovirus DNA in leukocytes by end-point titration and duplex polymerase chain reaction. J Virol Methods 1994; 49: 195–208.